(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 July 2002 (18.07.2002)

PCT

(10) International Publication Number
WO 02/054881 A2

(51) International Patent Classification[7]: A23C 9/12

(21) International Application Number: PCT/EP02/00438

(22) International Filing Date: 9 January 2002 (09.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01200069.1 10 January 2001 (10.01.2001) EP

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/054881 A2

(54) Title: THE USE OF FOOD AND DRINK AS A DELEVERY SYSTEM FOR PHYTASE IN HUMANS

(57) Abstract: The present invention relates to the use of phytase to increase the uptake of minerals, and in particular calcium, in a diet for humans. Advantageously milk is used as the delivery system for phytase for human consumption.

THE USE OF FOOD AND DRINK AS A DELIVERY SYSTEM FOR PHYTASE IN HUMANS

Filed of the invention

The present invention relates to the uptake of minerals such as calcium from food.

Background of the invention

Minerals such as Iron, Zinc and Calcium are important elements for human health. Iron deficiency results in anaemia which, in the case of pregnant women, is associated with significant increases in maternal mortality. Less severe anaemia results in a hampering of physical performance.

Iron needs in men and women have been assessed in terms of the amount of iron that must be absorbed to replace the body's losses and that needed to provide for normal body iron accretion rates during growth and pregnancy.

Zinc deficiency results in a depression in growth and severe skin lesions.

Calcium is very important in bone formation.

The availability of minerals such as Iron, Calcium and Zinc in animals and humans is hampered by the presence of compounds which form complexes with them. Phytic acid (inositol hexakisphosphate), for example, forms complexes with these minerals resulting in phytate salts. Phytates and phytic acid are found in virtually all plants and plant seeds. Phytic acid serves as a storage means for phosphorous source is plants.

Everyday foods such as bread, corn flakes and crackers contain considerable amounts of phytate and phytic acid. The consumption of such foods results in a reduced availability of minerals such as Iron, Zinc and Calcium.

Summary of the invention

Accordingly, the present invention provides a food or drink for human consumption, comprising a phytase.

The present invention further provides for:

- the use of a phytase in a food or drink for increasing the availability of Calcium, Iron and/or Zinc of the food or drink for humans.
- the use of a food or drink as delivery system for phytase for human consumption; and
- the use of a phytase in the manufacture of a medicament for treating a mineral deficiency.

Detailed description of the Invention

The present invention provides foods and drinks comprising phytases, which are suitable for human consumption.

Typically, the foods and drinks of the invention will comprise phytase at a concentration of from 50 to 10,000 FTU/kg, preferably from 100 to 5,000 FTU/kg and most preferably from 500 to 1,500 FTU/kg.

Phytases are widespread in nature and have been found in bacteria, yeasts, fungi and plants and phytases from any of these may be used in the invention. The fungal enzyme Phytase from *Aspergillus niger* has been commercialized for use in animal feed and may also be used in the present invention. The gene encoding the enzyme has been cloned and the phytase enzyme has been overexpressed in *Aspergillus niger*. This fungus is grown on industrial scale in large fermentors allowing for the production of the enzyme. The fungus secretes considerable amounts of phytase which can be separated from the biomass in a series of filtration and ultrafiltration steps. The resulting concentrated ultrafiltrate is subsequently formulated into a stable granulate or liquid which may be used in the present invention. Inclusion of the enzyme in the diet results in liberation of phosphate from phytic acid and phytate during passage of the diet through the gastro-intestinal tract of the animals.

The foodstuffs of the invention will typically be ones in which their preparation, storage or subsequent use do not involve conditions incompatible with phytase activity. The foods may be ones rich in phytic acid or phytates such as bread, cakes, pastries, breakfast cereals or crackers. The foods may also be enriched in minerals, in particular in Calcium, Zinc, and/or Iron and especially in Calcium. In a preferred embodiment the foods will be ones whose preparation do not involve heat treatments above 100°C and/or which are kept chilled prior to use.

We have found beneficial effects of phytase on the availability of Calcium, Iron and Zinc in humans when taken together with food containing these elements. The present invention describes a method to deliver the enzyme in a safe and attractive way to humans.

One way to deliver the phytase to humans is the uptake around or together with a meal, for example, pills which could be taken around eating time. However, since people like to reduce the number of pills they take to the minimum and since there is a chance of forgetting to take these pills. The preferred method of delivering the phytase is in the food or drink of a subject by adding the phytase to the food or drink. Hence another way to increase the availability of the essential elements comprises the processing of food with phytase to decrease the phytate content of the diet. Although, the invention provides for foods comprising phytases for some foods this may involve too large an effort from the food processing companies or would not work since the conditions under which the food is processed are incompatible with the conditions under which the enzyme would show activity. Accordingly, in a preferred embodiment the invention provides for drinks comprising a phytase.

The drink is typically tailored for human consumption in terms of taste and looks. The drink is preferably a flavoured drink and may be carbonated. Typically the drink is one which is kept chilled or refrigerated. The drink may be Calcium enriched.

The preferred way to deliver phytase to humans would be milk, preferably pasteurized cow's milk, since this is consumed every day by many people, is stored cool and contains a lot of Calcium. Alternatively goat or sheep milk may be used . Milk is frequently fortified with extra Calcium. This is practically useless if the milk is consumed simultaneously with bread or corn flakes containing high amounts of phytic acid or phytate since virtually all Calcium ions would be made unavailable due to the formation of Calcium phytate complexes. However, when a phytase is added this helps ensure the consumers derives full benefit from the additional calcium. The principle behind the present invention is believed to be the beneficial effects of the enzyme phytase on the availability of minerals such as Iron, Zinc and Calcium for uptake from food. Phytate and Phytic acid may be converted into inositol and inositol phosphates by the enzyme Phytase (for example, 3-phytase EC 3.1.3.8, 6-Phytase EC 3.1.3.8 or 3,6-Phytase EC 3.1.3.8 either of which may be employed in the invention). In this way the formation of complexes such as Calcium phytate complex can be substantially reduced and therefore

the minerals, in this example Calcium, is better available for uptake during consumption of food.

Milk is heat-treated to kill micro-organisms and to destroy undesirable enzyme activities. This heat treatment, termed pasteurization, is carried out at 60-85°C during a short period of up to 20 seconds. These temperatures in a watery environment are such that it would be expected that all phytase enzyme activity would be destroyed.

However, surprisingly we found that a large proportion of added phytase activity appears to be resistant to this heat treatment, whereas similar treatments in water result in a marked reduction in phytase activity. Milk represents a suitable means for delivering this enzyme to humans using milk as a functional food enriched with minerals and phytase.

The present invention also provides foods comprising or made with a milk of the invention such as cheeses, yoghurts, milk shakes, creams and desserts.

Although the milks of the invention are typically pasteurized the invention also provides unpasteurized milks comprising a phytase, as well as UHT milks comprising a phytase. The phytase may be added to the milk before, after or during pasteurization but preferably prior to pasteurization. If the phytase is added post pasteurization it is preferably added in a sterile form.

The food and drinks of the invention are typically suitable for human consumption in terms of their taste and appearance. They will be typically given to healthy individuals, usually as part of their normal diet. However, the food and drinks of the invention may be given to those suffering or at risk of mineral deficiency and may be given to treat, alleviate or prevent such deficiencies. They may be given to individuals suffering from anemia, calcium, zinc and/or iron deficiency. They may also be given as part of the normal diet of pregnant women or women recently having given birth. They may be given to men and women intending to conceive.

By Phytase is intended an enzyme that is capable of liberating at least one phosphate group from phytate.

The examples herein are give by way of illustration and are in no way intended to limit the scope of present invention. It will be obvious to those skilled in the art that phytase preparations used in this application may be obtained from various sources (of bacterial, fungal or plant origin).

Examples

Example 1

Application of phytase in milk prior to pasteurization

Various phytase preparations are commercially available. We have taken NATUPHOS™ 5000 G (DSM, Delft, The Netherlands) and the corresponding liquid formulation NATUPHOS™ L as well as the original concentrated ultrafiltrate as test substances.

We have compared the results of pasteurization during 20 seconds in cow's milk with those in water on phytase activity. Results, expressed as FTU/kg are shown in Table 1.

Phytase units were added to milk and water to a final concentration of 1000 FTU/kg. One phytase unit is defined as the amount of enzymes which liberates one micromole of phosphate per minute from 1mM Na-phytate at pH 5.5 at 37°C. The analytical method has been published (Engelen, van Ransdorp en Smit, J.A.O.C. Int. 77:760-764 (1994)).

Table 1: Phytase stability on milk and water

	Treatment			
	Milk 60°C FTU/kg	Milk 85°C FTU/kg	Water 60°C FTU/kg	Water 85°C FTU/kg
NATUPHOS™ 5000 G	995	925	553	275
NATUPHOS™ 5000 L	989	931	547	284
Phytase ultrafiltrate	997	936	549	263

CLAIMS

1. A food or drink for human consumption, comprising a phytase.

2. A food or drink according to claim 1, with a phytase content of from 50-10,000 FTU/kg, preferably from 100-5,000 FTU/kg or most preferably from 500-1,500 FTU/kg.

3. A food or drink according to claim 1 or 2, wherein the phytase is of microbial origin and is preferably from the fungus *Aspergillus*.

4. A food or drink according to any one of the preceding claims which is, or comprises, milk.

5. A food or drink according to claim 4, wherein the milk is pasteurized milk comprising a phytase, and preferably is pasteurized cow's milk.

6. Use of phytase in a food or drink for increasing the availability of Calcium, Iron and/or Zinc of the food or drink for humans.

7. Use of a food or drink as delivery system for phytase for human consumption.

8. Use according to claim 6 or 7, wherein the food or drink is a food or drink as defined in any one of claims 1 to 5.

9. Use of a phytase in the manufacture of a medicament for treating a mineral deficiency.

10. Use according to claim 9, wherein the food or drink is a food or drink as defined in any one of claims 1 to 5.

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WO 02/054881 A3

(51) International Patent Classification[7]: A23C 9/12, A23L 1/03, A61K 38/46

(21) International Application Number: PCT/EP02/00438

(22) International Filing Date: 9 January 2002 (09.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01200069.1 10 January 2001 (10.01.2001) EP

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(72) Inventor; and

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
30 October 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/00438

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23C9/12 A23L1/03 A61K38/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23C A23L A23K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 30681 A (OHMANN ANDERS ;NOVONORDISK AS (DK); KNAP INGE HELMER (DK)) 16 July 1998 (1998-07-16) claims 1-4,9 page 1, line 9 - line 30 page 9, line 14 - line 19 page 10, line 13 - line 18 page 15, line 11 - line 24 ---	1-3,6-9
X	EP 0 380 343 A (ALKO LTD) 1 August 1990 (1990-08-01) page 2, line 11 - line 16 page 3, line 45 - line 58 page 4, line 24 - line 27 page 10; claims 1,3 --- -/--	1,3,4, 6-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2002	18/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Inceisa, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/00438

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 49740 A (DSM NV ;HOF JAN BART (NL); KIES ARIE KARST (NL); BEUDEKER ROBERT F) 7 October 1999 (1999-10-07) page 9 -page 10; example 1; table 1 page 11 -page 12; example 2; table 3 page 3, line 20 - line 30 page 6, paragraph 2 page 8, line 19 - line 24 ---	1-3
X	DATABASE WPI Section Ch, Week 198444 Derwent Publications Ltd., London, GB; Class D13, AN 1984-272178 XP002167383 & JP 59 166049 A (NIPPON SHINYAKU CO LTD) abstract ---	1,6-9
X	DATABASE WPI Section Ch, Week 199519 Derwent Publications Ltd., London, GB; Class B04, AN 1995-143836 XP002212003 & JP 07 067635 A (AMANO PHARM KK), 14 March 1995 (1995-03-14) abstract ---	1,7-9
X	EP 0 682 876 A (SOUFFLET ALIMENTAIRE) 22 November 1995 (1995-11-22) page 3, line 17 - line 22 page 3, line 37 - line 38 ---	1,4,5,7, 8
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1992 TURK M ET AL: "PHYTATE DEGRADATION DURING BREADMAKING EFFECT OF PHYTASE ADDITION" Database accession no. PREV199294049988 XP002212002 abstract & JOURNAL OF CEREAL SCIENCE, vol. 15, no. 3, 1992, pages 281-294, ISSN: 0733-5210 -----	1,3-5,7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/00438

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 10
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 10

Dependent claim 10 has a wrong reference to claim 9. Claim 10 refers to a food or drink whereas claim 9 discloses a medicament.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/00438

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